Research Letter

Large-scale purification of functional recombinant human aquaporin-2

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Abstract The homotetrameric aquaporin-2 (AQP2) water channel is essential for the concentration of urine and of critical importance in diseases with water dysregulation, such as nephrogenic diabetes insipidus, congestive heart failure, liver cirrhosis and pre-eclampsia. The structure of human AQP2 is a prerequisite for understanding its function and for designing specific blockers. To obtain sufficient amounts of AQP2 for structural analyses, we have expressed recombinant his-tagged human AQP2 (HT-AQP2) in the baculovirus/insect cell system. Using the protocols outlined in this study, 0.5 mg of pure HT-AQP2 could be obtained per liter of bioreactor culture. HT-AQP2 had retained its homotetrameric structure and exhibited a single channel water permeability of $0.93 \pm 0.03 \times 10^{-13}$ cm^3/s, similar to that of other AQPs. Thus, the baculovirus/insect cell system allows large-scale expression of functional recombinant human AQP2 that is suitable for structural studies.

Key words: Baculovirus; Insect cell; Expression; Stopped-flow analysis; Structural analysis

1. Introduction

Water is the major constituent of all living cells and their environment. Therefore, water transport across cell membranes is crucial for all forms of life. Although water may cross lipid bilayers by simple diffusion, many physiological processes involving fluid transport require specialized water channels, called aquaporins (AQPs). These proteins belong to the family of the major intrinsic protein of the eye lens (MIP), which comprises some 200 members found in man, animals, plants and microorganisms [1]. In mammals, 10 AQPs have thus far been identified [2]. They are expressed in different tissues and are thought be involved in several processes of fluid transport, including osmoregulation and water conservation by the kidney, fluid secretion and absorption by epithelia of the gastrointestinal, respiratory and reproductive tracts and their associated exocrine glands, and the formation of cerebrospinal fluid, aqueous humor, amniotic fluid and sweat [2,3]. In the kidney, AQPs 1–4 and 6–8 are expressed, but at present the specific role in renal water conservation has only been established for AQPs 1–4 [4,5]. Of the 180 l of pro-urine produced daily, 90% is reabsorbed via AQP1, which is expressed in the basolateral and apical membranes of epithelial cells in the proximal tubules and descending limbs of Henle. Water reabsorption in the collecting duct is mediated through AQP2 in the apical membrane, and through AQP3 and AQP4 in the basolateral membrane, and is under the control of the anti-diuretic hormone vasopressin (AVP) [6–8]. After binding of AVP to its V2-receptor at the basolateral side of renal principal collecting duct cells, activation of cAMP-dependent protein kinase A (PKA) results in the redistribution of the homotetrameric AQP2 protein from intracellular storage vesicles to the apical plasma membrane, where it can exert its function as a water channel. Removal of AVP reverses this process, restoring the water-impermeable cell layer. AQP2 plays a critical role in regulating the body water balance: First, AQP2 is involved in congenital and acquired Nephrogenic Diabetes Insipidus, where the kidney is unable to concentrate urine in response to AVP [5,9–11]. Second, in diseases such as congestive heart failure [11–14], liver cirrhosis [14–17] and pre-eclampsia [11,14,18,19], the unrestricted water reabsorption via AQP2 may lead to life-threatening hyponatremia. Because AQP2 is directly involved in these disturbances of the body osmoregulation, rational design of specific AQP2 blockers is of prime clinical relevance. For this, elucidation of its 3D structure is an obligatory prerequisite.

Thus far the atomic structures of AQP1 and GlpF have been described, while 2D crystals of several AQPs have been produced. In addition, 3D crystals of AQP1 yielding diffraction to 2.2 Å have recently been reported [20]. Crystallization of all three of these proteins has been greatly aided by the availability of large amounts of highly pure protein. The bacterial channel proteins AqpZ and GlpF have been expressed as recombinant proteins in *Escherichia coli* and isolated using a poly-histidine tag [21,22]. MIP26 has been isolated from lenses, where it makes up 50–60% of the membrane proteins [23] and AQP1 has been purified from human and bovine erythrocytes in which it is expressed in high levels [24]. In contrast, AQP2 is only noticeably present in renal principal

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collecting duct cells [25] and testis [26], which renders large-scale isolation of AQP2 from natural sources impossible.

In the present study we expressed recombinant his-tagged human AQP2 in Sf9 insect cells, optimized the expression and purification of this recombinant protein, and functionally characterized it after reconstitution into lipid bilayers. Sufficient amounts of functional human AQP2 have been obtained and with this the basis has been laid for further studies on 2D crystallization and structural analysis of this protein.

2. Materials and methods

2.1. cDNA cloning and production of baculovirus

For the expression of AQP2 in Sf9 insect cells, the Bac-to-Bac baculovirus expression system (Gibco BRL) was used. Human AQP2 cDNA was amplified from an AQP2-containing pBluescript vector [10] using the primer AQP2-for-1 (ATGTGGGAGCTC-CGCTCCATAGC), corresponding to positions 1 to 23 of the human AQP2 coding sequence, and an extended T3 RNA polymerase reverse primer. The obtained PCR product of 860 bp was digested with *Hin*dIII and ligated into the pFastBac-HT-b vector, which was previously digested with *Sfo*I and *Hin*dIII. The resulting construct, pFB-HT-AQP2, encodes AQP2 with a poly-histidine tag and an rTEV protease cleavage site [27] flanking the N-terminus of AQP2 (Fig. 1). This construct was sequenced and subsequently transformed into competent DH10Bac *E. coli* cells, to allow transposon-directed recombination with the baculovirus genome. High molecular weight recombinant bacmid DNA was isolated from positive clones and transfected into Sf9 insect cells using the cellfectin reagent (Gibco BRL). Baculovirus stocks were obtained from the supernatant of these cultures after approximately 3 days, and titrated.

2.2. Expression of HT-AQP2

This procedure was adapted from the one optimized for recombinant his-tagged rhodopsin [28]. Sf9 cells were grown in a 15-l bioreactor (Applikon) containing 10 l of Insect Xpress medium (BioWhittaker), which was gently stirred at 80 rpm and kept at a constant 50% oxygen-saturation level and 27°C. Cells were grown to a density of $\sim 1.5\times10^6$ cells/ml, at which point the culture was infected with HT-AQP2 encoding baculovirus at an MOI of 0.05. A small sample was taken daily to follow expression of HT-AQP2. At day 5 or 6, when oxygen use of the culture had declined to zero, HT-AQP2 expression was at a maximum. At this point, cells were harvested by 10 min centrifugation at $5000\times g$ and 4°C.

2.3. Immunocytochemistry of Sf9 cells

Sf9 insect cells were grown on coverslips and infected with HT-AQP2 encoding baculovirus. Two days after infection, cells were fixed and permeabilized as described [29], and incubated with affinity-purified rabbit antibodies directed against the last 15 amino acids of rat AQP2 [30]. AQP2 was visualized using FITC-coupled goat α-rabbit IgG (Sigma) as secondary antibodies. Mock-infected Sf9 cells were used as a control. Images were taken with a Bio-Rad MRX-1000 confocal laser scanning microscope.

2.4. Immunoblotting

Protein samples were subjected to electrophoresis on a 13% SDS–polyacrylamide gel and immunoblotted onto PVDF membranes (Millipore) by standard procedures. Membranes were blocked for 1 h in 5% non-fat-dried milk in TBS-T (TBS with 0.1% Tween 20) and incubated overnight at 4°C with affinity-purified rabbit anti-AQP2 antibodies [30], diluted 1:3000 in TBS-T containing 1% non-fat-dried milk. Subsequently, blots were incubated for 1 h at RT with 1:5000-diluted goat α-rabbit IgG coupled to horseradish peroxidase. AQP2 was visualized using enhanced chemoluminescence (Pierce).

2.5. Urea/alkaline stripping of membranes

Urea/alkaline stripping was performed essentially as described [31]. Briefly, insect cells were centrifuged for 10 min at $3000\times g$ and homogenized in cold 5 mM Tris–HCl, 100 mM NaCl, pH 8.0 at 1×10^8 cells/ml by 20 strokes at 500 rpm in a Potter–Elvehjem tube. Membranes were pelleted by 30 min centrifugation at $100\,000\times g$ and 4°C. The membrane pellet was homogenized in 5 mM Tris, 1 mM EDTA, 4 M Urea, pH 8.0 as above and centrifuged for 45 min at $100\,000\times g$ and 4°C. The resulting pellet was homogenized in 20 mM NaOH (pH 12) and centrifuged for 90 min at $100\,000\times g$ and 4°C. The final pellet was subjected to two rounds of homogenization in 5 mM Tris, 100 mM NaCl, pH 8.0 and 30 min centrifugation at $100\,000\times g$ and 4°C, to neutralize the pH.

2.6. Determination of the oligomerization state of HT-AQP2

Urea/alkali-stripped membranes of HT-AQP2-expressing insect cells from 100 ml of culture were solubilized in 50 ml solubilization buffer (20 mM Tris, 300 mM NaCl, 1 mM L-histidine, 10% glycerol, 0.01% NaN_3, pH 8.0) containing 4% OG by 10 strokes at 500 rpm in a Potter–Elvehjem tube and overnight stirring at 4°C. After 60 min centrifugation at $100\,000\times g$ and 4°C, a 200-μl sample of solubilized protein was loaded onto a 4.0-ml 5–17.5% sucrose gradient in 20 mM Tris (pH 8.0), 5 mM EDTA, 0.1% Triton X-100 and centrifuged for 16 h at $150\,000\times g$. The position of the HT-AQP2 peak was determined by immunoblot analysis of 250-μl fractions taken from the gradient. Marker proteins were used to estimate the oligomeric size of HT-AQP2. Membranes of *Xenopus* oocytes expressing wild-type AQP2 [32] were solubilized in the same manner and taken as a control.

2.7. Purification of HT-AQP2

The membrane pellet of a 10-l expression culture of HT-AQP2 was urea/alkaline stripped and homogenized in solubilization buffer containing 4% OG (25 ml/l culture) by 20 strokes at 500 rpm in Potter–Elvehjem tubes. Membranes were solubilized by gentle overnight stirring at 4°C. Solubilized proteins were separated from insoluble fractions by 60 min centrifugation at $100\,000\times g$ and 4°C, and diluted by adding an equal volume of solubilization buffer. HT-AQP2 was bound to Ni–NTA by adding 400 μl of a 50% 'slurry' of Ni–NTA beads to 50 ml of solubilized proteins and gentle overnight stirring at 4°C. The beads with bound HT-AQP2 protein were centrifuged for 15 min at $4000\times g$ and 4°C, and washed once with solubilization buffer containing 2% OG. Pure HT-AQP2 was eluted by incubation with solubilization buffer containing 2% OG and 100 mM L-histidine (1 ml/l of original culture) during 2 h of gentle stirring at 4°C. After centrifugation, the HT-AQP2-containing supernatant was directly used for further analysis.

2.8. Functional analysis of purified HT-AQP2

Purified HT-AQP2 was reconstituted into proteoliposomes by mixing with *E. coli* lipids and overnight dialysis against liposome buffer (20 mM Tris–HCl, 50 mM NaCl, pH 7.2) at room temperature. Proteoliposomes were filtered through a 0.2-μm filter and diluted to 0.2 mg lipid/ml. The average vesicle diameter was calculated from electron micrographs of negatively stained vesicles [33]. For functional analysis, vesicles were subjected to an inwardly directed sucrose gradient of 100 mOsm/kg H_2O through rapid mixing in an Applied PhotoPhysics SX18.MV stopped-flow apparatus. The decrease of vesicle volume was measured from the increase in 90° scattered light at $\lambda = 450$ nm. Data from 10 measurements were averaged and light scattering was normalized at $t = 10$ s. The ability of mercuric compounds to inhibit HT-AQP2 water transport was investigated by the addition of 1 mM $HgCl_2$ 15 min prior to measurements. Time courses were fitted to single-exponential curves, allowing determination of the rate constant of HT-AQP2-facilitated water transport, k, by subtracting the rate constants of $HgCl_2$-treated vesicles from those of untreated vesicles. The osmotic water permeability coefficient (P_f in cm/s) was calculated according to the equation $P_f = k/((S/V_0)\cdot V_w\cdot(C_{out}-C_{in})\cdot\sigma)$, where (S/V_0) is the mean vesicle surface area to initial volume ratio, V_w is the partial molar volume of water, $(C_{out}-C_{in})$ is the difference in external and internal osmolarity, and σ is the reflection index of glucose ($\sigma_{glu} = 1$) [34]. The single channel water permeability (p_f) was calculated using the formula $p_f = P_f/\mathrm{SuD}$, where SuD is the single channel density per unit of surface area. SuD was calculated from the lipid to protein ratios (LPR) of the proteoliposomes (protein determination via amido-black 10B assay [35] and phospholipid determination via an ammonium ferrothiocyanate assay [36]) and an *E. coli* lipid surface density of 2.7 kDa/nm^2 [33]. Liposomes without protein were used as controls.

Protein:	N-terminus:
AQP2	MWELRSIAFSRAVFA
HT-AQP2	MSYYHHHHHHDYDIPTTENLYFQGMWELRSIAFSRAVFA
HT-AQP2 treated with rTEV protease	GMWELRSIAFSRAVFA

Fig. 1. Sequences of the N-termini of AQP2 proteins. The N-termini of wild-type AQP2, recombinant his-tagged AQP2 (HT-AQP2) and HT-AQP2 after treatment with rTEV protease are shown. The recognition sequence of rTEV protease is underlined.

3. Results

Using the Bac-to-Bac baculovirus expression system, a baculovirus was constructed that allows expression of AQP2 with a poly-histidine tag and an rTEV protease cleavage site flanking its N-terminus. Thus the HT-AQP2 can efficiently be purified from insect cell membranes by Ni-affinity chromatography. In addition, by treatment with rTEV protease, the recombinant HT-AQP2 can be reverted to a protein that only has an additional glycine at its N-terminus compared to the wild-type AQP2 protein (Fig. 1).

In collecting duct principal cells AVP promotes redistribution of AQP2 from intracellular vesicles to the plasma membrane. To locate the recombinantly expressed HT-AQP2 protein in insect cells, Sf9 cells infected with HT-AQP2 baculovirus at an MOI of 1 were subjected to immunofluorescent labeling 2 days after infection, and analyzed by confocal laser scanning microscopy. At this time point, where some but not yet all cells expressed HT-AQP2, specific staining for AQP2 was found in and close to the plasma membrane (Fig. 2). Mock-infected cells did not show any staining (not shown). This distribution was confirmed by cell fractionation experiments, which demonstrated HT-AQP2 to be present in similar amounts in both the plasma membrane and the vesicle fraction (not shown). Therefore, the total membrane fractions of insect cells were subsequently used for the purification of HT-AQP2.

To purify HT-AQP2 suitable for structural analyses it has to be solubilized from insect cell membranes by a detergent that (1) allows efficient binding of HT-AQP2 to Ni–NTA beads, (2) does not impair the structure of HT-AQP2, (3) conserves the functionality of HT-AQP2 as a water channel and (4) enables the reconstitution of HT-AQP2 into 2D crystals via dialysis or its assembly into 3D crystals. Of the detergents tested, 4% *n*-octyl-β-D-glucopyranoside (OG) appeared to be most effective during solubilization and Ni binding of HT-AQP2, although this detergent did require prior urea/alkaline stripping of the insect cell membranes for efficient solubilization.

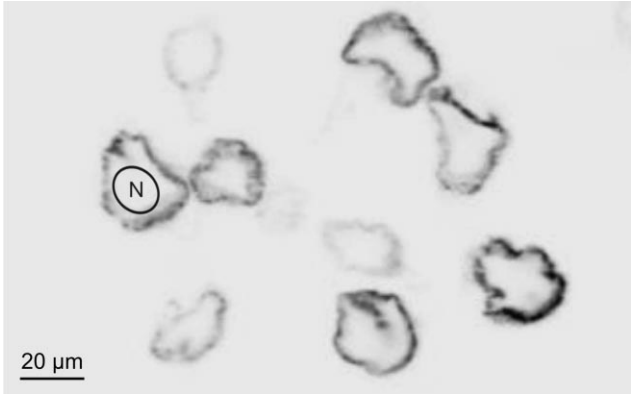

Fig. 2. Localization of HT-AQP2 in infected Sf9 insect cells. Confocal laser scanning microscopy revealed plasma membrane and intracellular vesicular staining after immunocytochemical analysis for AQP2. Relative size and position of an insect cell nucleus is indicated in one cell (N).

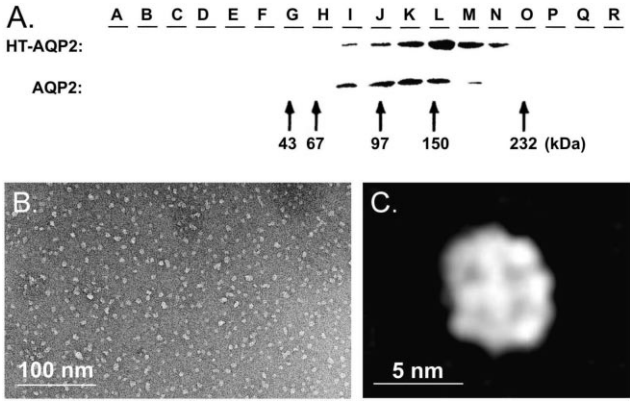

Fig. 3. Determination of the oligomeric size of HT-AQP2. Insect cell membranes containing HT-AQP2 were solubilized in 4% OG and subjected to sucrose gradient centrifugation (A). Fractions from this sucrose gradient (A to R) were subjected to immunoblotting for AQP2. Peak fractions of marker proteins are indicated by arrows and their molecular masses in kDa. Recombinant wild-type AQP2 was used as a control. Negatively stained solubilized HT-AQP2 particles were also analyzed by means of electron microscopy (B) and averaged (C) to show the tetrameric structure of the protein after solubilization in OG.

As shown for other AQPs [21,37–40], in vivo and when heterologously expressed in *Xenopus* oocytes, AQP2 is a homotetramer [32]. To test whether HT-AQP2 expressed in insect cells is a homotetramer as well that survives solubilization with OG, HT-AQP2 was solubilized from stripped insect cell membranes with 4% OG and analyzed by sucrose gradient centrifugation. Wild-type AQP2 expressed in *Xenopus* oocytes served as a control. As can be seen in Fig. 3A, immunoblotting of fractions from the sucrose gradient revealed that HT-AQP2 peaked in the fraction running at 150 kDa [L], while wt-AQP2 was most abundant in the fraction running at a slightly lower molecular mass [K]. Since the molecular mass of HT-AQP2 is 32 kDa and that of wild-type AQP2 is 29 kDa, the sedimentation measurements are compatible with tetrameric structures for both proteins. The tetrameric organization of HT-AQP2 was further confirmed by negative staining electron microscopy of solubilized HT-AQP2 protein (Fig. 3B), followed by single particle analysis using the Semper program [41]. The resulting average image of 162 HT-AQP2 particles (Fig. 3C) clearly shows a tetrameric structure for this protein. Therefore, HT-AQP2 expressed in Sf9 insect cells appears to be correctly and stably folded, allowing its tetrameric structure to endure solubilization with 4% OG.

For future crystallization experiments, HT-AQP2 has to be purified from large bioreactor expression cultures to obtain sufficient amounts of protein. At an MOI of 0.05, used in such cultures [28], the expression of HT-AQP2 started around day 3 and reached a maximum 5 to 6 days after infection. Interestingly, this coincided with the rise and fall of oxygen use of the cell culture (not shown). Depending on the individual expression profile, cells were therefore harvested at day 5 or 6. HT-AQP2 was purified according to the procedure de-

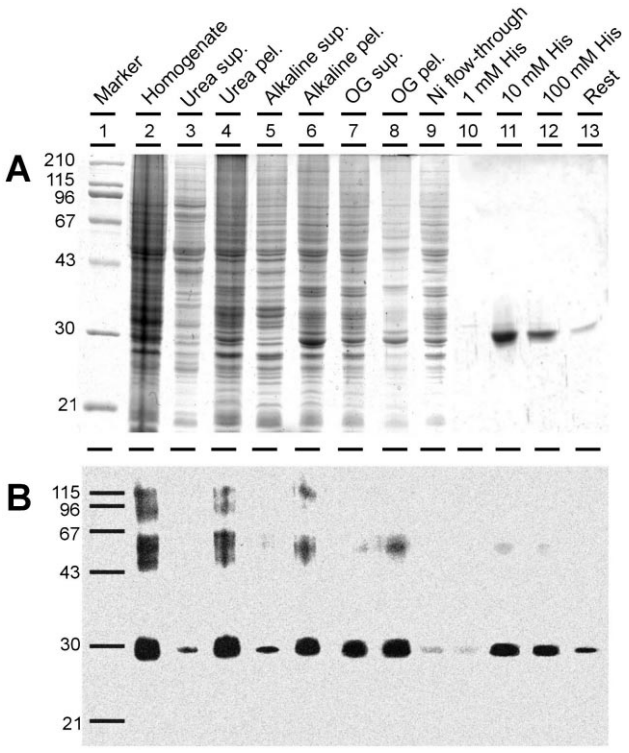

Fig. 4. Purification of HT-AQP2 from large bioreactor culture. Insect cell membranes containing HT-AQP2 were urea/alkaline stripped and solubilized in OG. HT-AQP2 was then purified via Ni-affinity chromatography. Samples were taken from all steps during this procedure to establish the purity of the product after each step by Coomassie-staining of samples loaded on a gel (A) and to estimate the yield of each step by immunoblotting the samples for AQP2 (B). The last four lanes of the Coomassie-stained gel, showing elution of HT-AQP2 from Ni–NTA beads, are 100× more concentrated than the previous lanes in order to visualize the HT-AQP2 protein. Higher bands in immunoblot most likely represent cross-linked products.

scribed above. The level of purification in each step was analyzed by PAGE-separation followed by Coomassie-staining (Fig. 4A) or immunoblotting (Fig. 4B). Urea/alkaline stripping (Fig. 4A, lanes 3 through 6) was found to efficiently remove contaminating proteins, while only a very small amount of HT-AQP2 protein (running at ~30 kDa) was lost (Fig. 4B, same lanes). With 4% OG, about 50% of the HT-AQP2 protein could be solubilized from the stripped membranes (Fig. 4B, lanes 7 and 8). Ni-affinity binding and subsequent elution of HT-AQP2 proved to be very efficient, since hardly any HT-AQP2 was found in the Ni–NTA flow-through (Fig. 4B, lane 9) while nearly all HT-AQP2 eluted from the Ni–NTA beads at 10- and 100-mM concentrations of L-histidine (Fig. 4B, lanes 10 through 13). The eluted HT-AQP2 appeared to be very pure (Fig. 4A, lanes 10 through 13). Approximately 0.5 mg of pure HT-AQP2 protein (out of a total of 1.6 mg HT-AQP2 initially expressed) could be obtained for each liter of insect cell culture, corresponding to a yield of ~37%.

Table 1
Overview of measured and calculated vesicle properties

	LPR 60	LPR 90
d_v	$0.74 \pm 0.02 \times 10^{-5}$ cm	$0.60 \pm 0.01 \times 10^{-5}$ cm
d_{em}	$1.04 \pm 0.03 \times 10^{-5}$ cm	$0.85 \pm 0.02 \times 10^{-5}$ cm
k	18.8 ± 0.8 s^{-1}	13.1 ± 0.6 s^{-1}
SuD	1.4113×10^{11} cm^{-2}	7.6075×10^{10} cm^{-2}
P_f	$1.29 \pm 0.06 \times 10^{-2}$ cm/s	$0.72 \pm 0.03 \times 10^{-2}$ cm/s
p_f	$0.91 \pm 0.04 \times 10^{-13}$ cm^3/s	$0.95 \pm 0.04 \times 10^{-13}$ cm^3/s

Average spherical vesicle diameters (d_v) were calculated from the average diameters obtained from electron micrographs (d_{em}) of negatively stained proteoliposomes (LPR 60, $n = 87$; LPR 90, $n = 51$). Rate constants of HT-AQP2 facilitated water transport (k) were calculated by subtracting the rates of $HgCl_2$-treated vesicles from those of the corresponding untreated vesicles. SuD, P_f and p_f were calculated as described in Section 2.

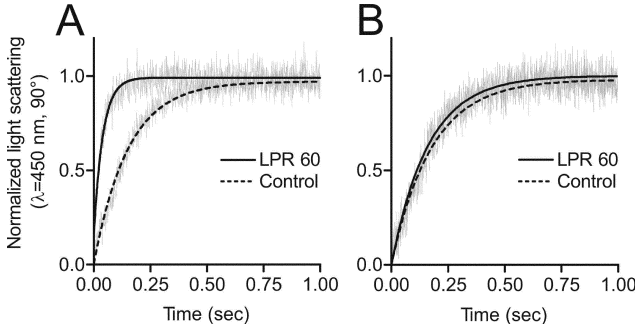

Fig. 5. Stopped-flow analysis of HT-AQP2 reconstituted in proteoliposomes. Proteoliposomes containing HT-AQP2 were subjected to an inwardly directed osmotic gradient by rapid mixing in a stopped-flow spectrometer, and vesicle shrinkage was followed by measuring the increase in scattered light. Graphs show the average time course ($n = 10$) of HT-AQP2-containing proteoliposomes at an LPR of 60 compared to empty control liposomes. All data were fitted to single-exponential curves. Water transport was measured without (A) or with the addition of 1 mM $HgCl_2$ (B), an inhibitor of AQP function.

The functionality of our purified HT-AQP2 was assessed with proteoliposomes whose osmotically driven shrinkage was measured by stopped-flow light scattering. Vesicle shrinkage of proteoliposomes containing HT-AQP2 appeared to occur much faster than with control liposomes containing no protein (Fig. 5A). Upon treatment of proteoliposomes with 1 mM $HgCl_2$, however, the HT-AQP2-facilitated water transport was completely reduced to control levels (Fig. 5B), further indicating that the increased osmotic shrinkage of HT-AQP2-containing vesicles was completely AQP-mediated. From the rate constants of the fitted curves and the average diameter of the proteoliposomes (Table 1), the single channel water permeability coefficient (p_f) of HT-AQP2 was determined to be $0.91 \pm 0.04 \times 10^{-13}$ cm^3/s at an LPR of 60, and $0.95 \pm 0.04 \times 10^{-13}$ cm^3/s at an LPR of 90. From these measurements, the resulting average p_f of HT-AQP2 equals $0.93 \pm 0.03 \times 10^{-13}$ cm^3/s.

4. Discussion

To lay the foundation for crystallization and structural analysis of AQP2, which is required for understanding the function of this water channel and for rational design of specific AQP2 blockers, high amounts of pure AQP2 needed to be produced. Since the amount expressed in renal collecting ducts is by far not sufficient, AQP2 needs to be heterologously expressed. As also shown by others [42] and as can be expected for eukaryotic integral membrane proteins, AQP2 could not be expressed in *E. coli*. As demonstrated in this

study, however, expression in the baculovirus/insect cell expression system resulted in large amounts of HT-AQP2 (i.e. 1.6 mg/l of culture). This level of expression was slightly lower than reported for AQP4 in the same system (2.1 mg/l of culture; [43]).

While prior urea/alkaline stripping of the membranes was essential for efficient solubilization, the coupled poly-histidine tag proved to be convenient for the isolation of HT-AQP2 from insect cell membranes (Fig. 4). It allowed a 37% recovery (i.e. 0.5 out of 1.6 mg) of highly pure HT-AQP2. In contrast, only 5% (0.11 mg/l of culture) of non-tagged AQP4 could be isolated from insect cells [43]. A his-tagged version of AQP4 in the same study had a better yield, however, in contrast to the recombinant wild-type AQP4 it appeared to have no water transport activity [43]. Therefore, analysis of the oligomeric structure and function of an isolated overexpressed membrane protein is essential.

As shown before for human and rat renal AQP2 and human AQP2 heterologously expressed in *Xenopus* oocytes [32], sucrose gradient sedimentation analysis and subsequent immunoblotting revealed that HT-AQP2 existed as a homotetramer (Fig. 3), indicating that the poly-histidine tag did not disturb the formation of the normal quaternary structure of AQP2. This was confirmed by electron microscopy of negatively stained HT-AQP2 preparations. Finally, stopped-flow light scattering of purified reconstituted HT-AQP2 (Fig. 5) yielded a single channel water permeability coefficient of $0.93 \pm 0.03 \times 10^{-13}$ cm^3/s, which is in between that of AQP4 (1.5×10^{-13} cm^3/s; [43]) and that of AQP1 (0.5×10^{-13} cm^3/s; [33]). These results indicate that in HT-AQP2 the poly-histidine tag does neither impair correct folding and tetramerization of the protein, nor the function of the water pore.

In conclusion, we have been able to isolate high amounts of structurally and functionally intact human AQP2, which is now available for crystallization and structural analyses. The structure of HT-AQP2 will provide clues to understanding functionality and regulation of AQP2, and will pave the road for rational design of AQP2 blockers that might ultimately be useful as AQP2-specific diuretics in patients suffering from congestive heart failure, liver cirrhosis and pre-eclampsia.

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